[CONTRIBUTION FROM THE THOMPSON CHEMICAL LABORATORY, WILLIAMS COLLEGE, WILLIAMSTOWN, MASS.]

The Synthesis and Antibacterial Activity of Analogs of Citrinin and Dihydrocitrinin

BY HAROLD H. WARREN, MANUEL FINKELSTEIN AND DANIEL A. SCOLA

RECEIVED DECEMBER 4, 1961

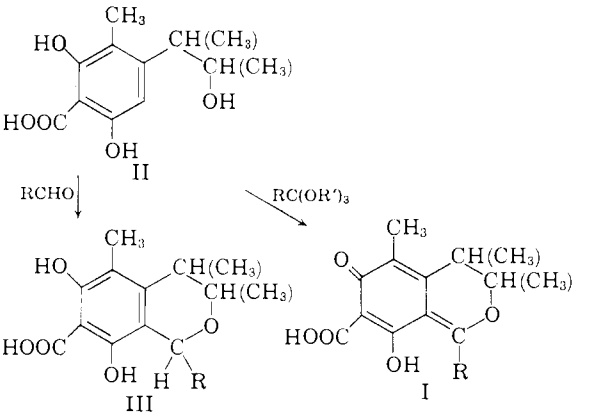
A general method for the synthesis of 1-alkylcitrinins (I) by the condensation of orthoesters with the carboxylic acid derivative of compound A (II) has been developed. Series of 1-alkylcitrinins and of 1-alkyldihydrocitrinins (III) ranging from 1-propyl to 1-nonyl have been prepared and their antibacterial activity measured. Progressive lengthening of the chain causes a marked increase in activity which exceeds that of citrinin in the C_6 and higher derivatives.

A general method for the synthesis of 1-substituted derivatives of citrinin (I) and of dihydrocitrinin (III) and the application of the method to the preparation of simple aryl and alkyl derivatives have been previously reported.[1,2] It was found that the introduction of a methyl group into citrinin causes nearly complete loss of antibiotic activity while slight activity was noted in the ethyl derivative and appreciable activity in the phenyl derivative, though considerably less than that of the parent compound. In the case of dihydrocitrinin the parent compound is inactive as are the 1-methyl, 1-ethyl and 1-phenyl derivatives, while the 1-benzyl derivative shows moderate activity compared to citrinin.

The present work was undertaken to determine whether the appearance of activity in 1-benzyldihydrocitrinin and its reappearance in 1-ethyl- and 1-phenylcitrinin is a function of the specific nature of the substituent or merely of the size or chain length. A homologous series of 1-*n*-alkyl derivatives of dihydrocitrinin ranging from the 1-propyl to 1-nonyl has been successfully prepared by the condensation of the appropriate normal aliphatic aldehydes with the carboxylic acid derivative of compound A (II)[3] (hereinafter referred to as compound F). All of these compounds are soluble in dilute aqueous sodium bicarbonate with evolution of carbon dioxide and give the characteristic deep blue color with ferric chloride in dilute alcohol. The properties of the compounds are summarized in Table I.

The conversion of the dihydrocitrinin derivatives to the corresponding citrinin derivatives by oxidation was less successful. Only the 1-hexyl derivative was obtained in satisfactory yield by use of bromine; other oxidizing agents or catalytic dehydrogenation were equally ineffectual.

A possible route to the 1-alkylcitrinins was suggested by the work of Gore[4] who accomplished a partial synthesis of citrinin by the condensation of compound F with ethyl orthoformate. We have found that aliphatic orthoesters in general will undergo a cyclization reaction with compound F forming 1-alkylcitrinins. The reaction occurs readily and completely at room temperature when compound F is dissolved in an excess of the orthoester. Rapid development of the lemon-yellow color characteristic of citrinin is followed in some cases by the spontaneous crystallization of the product. Use of ethyl acetate as a solvent permits a decrease in the amount of orthoester required and insures a homogeneous reaction mixture. No reaction occurs in ethanol.



That the product of this reaction is indeed the predicted substituted citrinin was demonstrated by comparing the condensation product of ethyl orthopropionate with authentic 1-ethylcitrinin produced by the oxidation of 1-ethyldihydrocitrinin. The compounds were identical in all properties, and the melting point of a mixture showed no depression.

By means of this reaction a series of 1-*n*-alkylcitrinins ranging from 1-propyl to 1-nonyl has been prepared. The compounds and their properties are summarized in Table II. Each of the compounds gives the characteristic red-brown color with ferric chloride in dilute alcohol. Because of their low water solubility the higher homologs do not dissolve readily in aqueous sodium bicarbonate but do dissolve in sodium bicarbonate in dilute ethanol and are not reprecipitated by dilution with water. Immediate precipitation occurs upon acidification.

A semi-quantitative determination of the antibacterial activity of the compounds toward four representative organisms was made using the agar streak technique of Waksman and Reilly.[5] As in the case of citrinin itself none of the compounds inhibits the growth of the gram-negative organism, *E. coli* at a maximum concentration of 3000 dilution units (ml. of nutrient agar/g. of test compound). All of the compounds are active in varying degree toward *S. aureus*, *B. mycoides* and *B. subtilis*. The results are summarized in Table III.

It is apparent that in the citrinin derivatives while introduction of a methyl group virtually

(1) H. H. Warren, G. Dougherty and E. S. Wallis, *J. Am. Chem. Soc.*, **71**, 3422 (1949).

(2) H. H. Warren, G. Dougherty and E. S. Wallis, *ibid.*, **79**, 3812 (1957).

(3) 4-(2-Hydroxy-1-methylpropyl)-3-methyl-γ-resorcylic acid.

(4) T. S. Gore, R. V. Talavdekar, and K. Venkataraman, *Current Sci. (India)*, **19**, 20 (1950); *C. A.*, **44**, 7313 (1950).

(5) S. A. Waksman and H. C. Reilly, *Anal. Chem.*, **17**, 556 (1945).

TABLE I

PRODUCTS OF THE REACTION OF COMPOUND F WITH ALIPHATIC ALDEHYDES

Product, dihydrocitrinin	R	Aldehyde	Yield anal. sample, %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1-Propyl-	$-(CH_2)_2CH_3$	Butanal	14	166.4–167.0 d.	$C_{16}H_{22}O_5$	65.31	65.19	7.54	7.62
1-Butyl-	$-(CH_2)_3CH_3$	Pentanal	17	157.4–158.8 d.	$C_{17}H_{24}O_5$	66.21	66.30	7.84	7.95
1-Pentyl-	$-(CH_2)_4CH_3$	Hexanal	7	145.4–146.2 d.	$C_{18}H_{26}O_5$	67.06	67.33	8.13	8.10
1-Hexyl-	$-(CH_2)_5CH_3$	Heptanal	28	141.5–142.2 d.	$C_{19}H_{28}O_5$	67.84	68.02	8.39	8.26
1-Heptyl-	$-(CH_2)_6CH_3$	Octanal	30	124.0–124.8	$C_{20}H_{30}O_5$	68.55	68.85	8.63	8.48
1-Octyl-	$-(CH_2)_7CH_3$	Nonanal	41	116.2–117.2	$C_{21}H_{32}O_5$	69.19	68.74	8.85	8.72
1-Nonyl-	$-(CH_2)_8CH_3$	Decanal	42	108.6–109.8	$C_{22}H_{34}O_5$	69.80	69.94	9.05	8.90

TABLE II

PRODUCTS OF THE REACTION OF COMPOUND F WITH ORTHOESTERS

Product, citrinin	Ethyl orthoester	R	Yield anal. sample, %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1-Propyl-	Orthobutyrate	$-(CH_2)_2CH_3$	66	125.4–126.4	$C_{16}H_{20}O_5$	65.75	65.83	6.90	6.89
1-Butyl-	Orthovalerate	$-(CH_2)_3CH_3$	44	104.0–104.6	$C_{17}H_{22}O_5$	66.65	66.48	7.24	7.37
1-Pentyl-	Orthocaproate	$-(CH_2)_4CH_3$	47	93.4–94.5	$C_{18}H_{24}O_5$	67.48	67.63	7.55	7.58
1-Hexyl-	[a]	$-(CH_2)_5CH_3$	30	117.7–118.6	$C_{19}H_{26}O_5$	68.24	68.62	7.84	7.50
1-Heptyl-	Orthocaprylate	$-(CH_2)_6CH_3$	49	75.6–76.6	$C_{20}H_{28}O_5$	68.94	69.09	8.10	8.20
1-Octyl-	Orthopelargonate[b]	$-(CH_2)_7CH_3$	44	57.7–58.6	$C_{21}H_{30}O_5$	69.57	69.76	8.34	8.48
1-Nonyl-	Orthocaprate	$-(CH_2)_8CH_3$	32	51.0–51.5	$C_{22}H_{32}O_5$	70.17	70.06	8.57	8.48

[a] Prepared by the oxidation of 1-hexyldihydrocitrinin. [b] Methyl ester.

TABLE III

ANTIBACTERIAL ACITIVITY OF CITRININ AND DIHYDROCITRININ DERIVATIVES

Compound	*S. aureus* Ml. agar/g. cpd. for complete inhib.	*B. mycoides* Ml. agar/g. cpd. for complete inhib.	*B. subtilis* Ml. agar/g. cpd. for complete inhib.
Citrinin	40,000	40,000	40,000
1-Phenyl-[a]	[b]	[b]	[b]
1-Methyl-[a]	...[c]	...	...
1-Ethyl-[a]	3,000	3,000	3,000
1-Propyl-	6,000	10,000	6,000
1-Butyl-	17,000	17,000	17,000
1-Pentyl-	50,000	35,000	35,000
1-Hexyl-	70,000	70,000	70,000
1-Heptyl-	70,000	100,000	70,000
1-Octyl-	70,000	100,000	70,000
1-Nonyl-	70,000	100,000	100,000
Dihydrocitrinin	...	...	...
1-Phenyl[a]	[b]	...	...
1-Benzyl[a]	6,000	6,000	6,000
1-Methyl-[a]	...	...	...
1-Ethyl-[a]	...	...	...
1-Propyl-	...	...	3,000
1-Butyl-	3,000	6,000	6,000
1-Pentyl-	10,000	25,000	17,000
1-Hexyl-	40,000	70,000	50,000
1-Heptyl-	70,000	70,000	100,000
1-Octyl-	100,000	150,000	100,000
1-Nonyl-	100,000	150,000	100,000

[a] Previously prepared[2] and included for comparison. [b] Partial inhibition of growth at 3,000 dilution units. [c] .... indicates no inhibition at highest concentration tested.

eliminates activity, there is an increase in activity with increasing chain length of the substituent. The activity approaches that of the parent compound at C_5, surpasses it and reaches a plateau at C_7 to C_9 at a value more than twice that of citrinin. The effect of a C_6-normal alkyl chain is considerably greater than that of the C_6-aromatic ring.

The marked decrease in antibacterial activity caused by introduction of a methyl group followed by the gradual enhancement of activity as the substituent chain length is increased suggests a new and different mode of action in the homologs that is less structurally specific than that of the parent compound and that is largely a function of the hydrophobic nature of the substituent.

In the case of the dihydrocitrinin derivatives, substituents of increasing chain length cause the appearance of activity at C_3 and a gradual increase to a plateau at C_8 to C_9 where the activity is more than double that of citrinin and somewhat higher than that of the corresponding citrinin derivatives. Here, as in the citrinin derivatives, the activity is a function of chain length. The influence of the phenyl or benzyl group is much less than that of the corresponding C_6- or C_7-normal alkyl group. This effect of substitution of dihydrocitrinin, which may be regarded as a substituted resorcinol, parallels the well known behavior of alkylresorcinols and other phenolic compounds,[6] the antiseptic action of which increases progressively as the chain length, and hence hydrophobic character of the substituent, is increased. In such compounds maximum activity is reached at some optimum chain length. Further lengthening of the chain causes a decrease in activity. Whether such is the case for the alkyldihydrocitrinins is now being studied.

Experimental[7]

Materials.—All of the aldehydes were commercially available and were redistilled before use. Compound F was prepared as previously described.[1,2] Ethyl orthopropionate and ethyl orthovalerate were commercially available. Ethyl orthobutyrate, methyl orthopelargonate, ethyl orthocaproate, ethyl orthocaprylate and ethyl orthocaprate

(6) See, for example, W. A. Sexton, "Chemical Constitution and Biological Activity," D. van Nostrand Co., Inc., New York, N. Y., 1950, Chapters 5, 16.

(7) All melting points are corrected. Decomposition occurs at the melting point of many of the compounds causing variation of the melting point with the rate of heating.

were prepared by the method of McElvain.[8,9] Of these compounds the last three have not been reported in the literature. Fractions were collected over the following boiling ranges: Ethyl orthocaprate, 136–138° (6 mm.); ethyl orthocaprylate, 215–225°; ethyl orthocaproate, 180–189°. Each fraction was contaminated with the corresponding normal ester and amide, but all reacted readily with compound F and were used without further purification or characterization.

The zinc chloride used was J. T. Baker, reagent grade.

1-Ethylcitrinin (**1-Ethyl-4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic Acid**).—A mixture of 1.0 g. of compound F and 2.0 ml. of ethyl orthopropionate was warmed briefly to give a homogeneous solution. A yellow color developed immediately. After standing for 5 min. at room temperature the solution gave a red-brown color with 5% aqueous ferric chloride. Evaporation of the excess orthoester produced a crystalline residue that after two recrystallizations from ethanol yielded 0.90 g. (77%) of lemon-yellow crystals melting at 138.2–139.2°, resolidifying and remelting at 265–268° dec. A mixture of this product with a sample of 1-ethylcitrinin produced by oxidation of 1-ethyldihydrocitrinin melted without depression of the melting point.

Anal. Calcd. for $C_{15}H_{18}O_5$: C, 64.73; H, 6.52. Found: C, 64.86; H, 6.57.

1-Alkylcitrinins (**1-Alkyl-4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic Acid**).—The following general procedure was found most satisfactory for the preparation of the 1-alkyl derivatives of citrinin. To a solution of 1.0 g. (0.0042 mole) of compound F dissolved in 15 ml. of hot ethyl acetate and cooled to room temperature was added 2.0 ml. of the appropriate orthoester. A yellow color developed immediately and gradually darkened. At the end of 15–30 min. the solution gave a red-brown color with 5% aqueous ferric chloride, indicating complete reaction of compound F. After 2 hours the solvent was evaporated on a steam-bath whereupon the residue crystallized spontaneously or when stirred with a little ethanol. Three or four recrystallizations from hot ethanol followed by drying under vacuum over phosphorus pentoxide at elevated temperature yielded a product suitable for analysis. The compounds, their properties, and analytical data are summarized in Table II.

In the case of 1-octylcitrinin and 1-nonylcitrinin, because of their relatively high solubility in the residual orthoester and their low melting points, it was found advantageous to extract them from the reaction mixture with dilute aqueous base. Dilute sodium hydroxide was used for the 1-octyl compound while dilute ammonium hydroxide was found more satisfactory for the 1-nonyl because of the low solubility of its sodium salt. The basic solution was acidified and extracted with ether. Evaporation of the ether after the solution had been dried over anhydrous sodium sulfate yielded a residue that was recrystallized as described above. Methanol proved to be a more effective solvent for 1-nonylcitrinin.

1-Hexylcitrinin (**1-Hexyl-4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic Acid**).—The oxidation of 1-hexyldihydrocitrinin was carried out successfully and was used in lieu of the orthoester synthesis. To a solution of 0.5 g. (0.0015 mole) of 1-hexyldihydrocitrinin in 15 ml. of chloroform was added dropwise 3.0 ml. of 0.5 *M* bromine in chloroform. After 1 hr. at room temperature the solution was evaporated to an oil which crystallized when stirred with ethanol. Three recrystallizations from ethanol yielded lemon-yellow crystals of sufficient purity for analysis. Properties and analytical data are given in Table II.

1-Alkyldihydrocitrinins (**1-Alkyl-6,8-dihydroxy-3,4,5-trimethyl-7-isochromancarboxylic Acid**).—The following general procedure was used in the preparation of all of the 1-alkyldihydrocitrinin derivatives. To a suspension of 2.0 g. (0.0083 mole) of compound F in 20 ml. of benzene at room temperature was added 4.0 ml. of the appropriate freshly distilled aldehyde and 0.4 g. of zinc chloride. Anhydrous hydrogen chloride was bubbled through for 30 sec. The suspended material dissolved within about 5 min. After standing for 2 hr. the solution was washed twice with water and dried over anhydrous sodium sulfate. Evaporation of the benzene yielded an oil that crystallized when stirred persistently with petroleum ether or with petroleum ether and ethylene bromide.

For the 1-propyl, 1-butyl, 1-pentyl and 1-hexyl derivatives three or four recrystallizations from benzene and one from 10% ethyl acetate–cyclohexane followed by drying over phosphorus pentoxide under vacuum at an elevated temperature yielded products suitable for analysis. The 1-heptyl and 1-nonyl compounds were recrystallized four times from benzene with addition of petroleum ether, and the 1-octyl derivative was recrystallized once from benzene and three times from *n*-heptane. The properties of the compounds and analytical data are summarized in Table I.

Assay for Antibacterial Activity.—The method of Waksman and Reilly[5] as previously described[2] was used. Compounds to be tested were converted to their sodium salts by dissolving them in the minimum of 5% sodium hydroxide with addition of a few drops of acetone and diluting to the appropriate concentrations. The stock solutions were used immediately to prevent possible decomposition of the compounds. The results are summarized in Table III.

(8) S. M. McElvain and J. W. Nelson, *J. Am. Chem. Soc.*, **64**, 1825 (1942).

(9) S. M. McElvain, R. E. Kent and C. L. Stevens, *ibid.*, **68**, 1922 (1946).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE 39, MASS.]

Proximity Effects. XXIII. Synthesis of the Seven Bicyclo[4.2.0]octanols[1,2]

BY ARTHUR C. COPE AND ROBERT W. GLEASON[3]

RECEIVED AUGUST 2, 1960

The preparation of the seven bicyclo[4.2.0]octanols is described and on the basis of the methods of synthesis, rates of oxidation, conformational analysis and infrared spectra, configurations have been assigned to five of them. *endo*-Bicyclo[4.2.0]octan-7-ol was found to be the bicycloöctanol formed in about 0.1% yield by the treatment of *cis*-cycloöctene oxide with formic acid. It was discovered that the lithium aluminum hydride reduction of bicyclo[4.2.0]oct-7-ene oxide gives in addition to the expected bicyclo[4.2.0]octan-7-ol, a monocyclic alcohol identified as *cis*-2-methylcyclohexanemethanol.

Among the "abnormal" products that have been isolated from reactions of cycloöctane derivatives are several bicycloöctanols. Bicyclo[3.3.0]octan-2-ols are formed on treatment of *cis*- and *trans*-cycloöctene oxides with lithium diethylamide and with phenyllithium,[4] on solvolysis of *cis*- and *trans*-1,2-cycloöctanediol ditosylates and *cis*-1,4-cycloöctanediol ditosylate[5] and on the solvoly-

(1) Supported in part by a research grant (NSF-G5055) of the National Science Foundation.

(2) Paper XXII, A. C. Cope, G. A. Berchtold, P. E. Peterson and S. H. Sharman, *J. Am. Chem. Soc.*, **82**, 6370 (1960).

(3) National Science Foundation Summer Fellow, 1959.

(4) A. C. Cope, H. H. Lee and H. E. Petree, *J. Am. Chem. Soc.*, **80**, 2849 (1958).

(5) A. C. Cope, S. Moon and P. E. Peterson, *ibid.*, **81**, 1650 (1959).